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

HALL, Anna <http://orcid.org/0000-0002-1491-7309>, FAIRCLOUGH, Andrew, MAHADEVAN, Kritika and PAXMAN, Jenny <http://orcid.org/0000-0003-3596-489X>

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Corresponding Author: Miss Anna C Hall, Bsc (Hons); MSc

Corresponding Author's Institution: Sheffield Hallam University

First Author: Anna C Hall, Bsc (Hons); MSc

Order of Authors: Anna C Hall, Bsc (Hons); MSc; Andrew C Fairclough, PhD; CBiol; MIBiol; MIFST; Kritika Mahadevan, BSc; MSc; PhD; MIFST; Jenny R Paxman, BA(Hons); MMedSci; PGCLT; MPhil; RNutr

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.

Suggested Reviewers: Tom Sanders BSc PhD DSc RPHNutr
Professor of Nutrition & Dietetics, Department of Nutrition & Dietetics, Kings College London
tom.sanders@kcl.ac.uk

Martin Yeomans PhD CPsychol AFBPsS
Professor of Experimental Psychology, School of Psychology, University of Sussex
martin@sussex.ac.uk

Jeffrey Pearson
Professor of Molecular Physiology, Institute for Cell and Molecular Biosciences, Newcastle University
j.p.pearson@ncl.ac.uk

Centre for Food Innovation

Stoddart Building

Sheffield Hallam University

Sheffield

S1 1WB

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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,

Anna Hall

BSc(Hons), MSc in Nutrition and Public Health Management (Sheffield Hallam University)

Lecturer in Public Health Nutrition at Sheffield Hallam University

Centre for Food Innovation

Sheffield Hallam University

Anna.hall@shu.ac.uk

Tel: +44 (0) 114 2256279

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.

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

Hall, AC[a]. Fairclough, AC[a]. Mahadevan, K[ab]. and Paxman, JR[a].

[a] Centre for Food Innovation, Sheffield Business School, City Campus, Howard Street, Sheffield, S1 1WB. United Kingdom.

[b] Present Address: Manchester Metropolitan University, Department of Food and Tourism Management, Manchester, M15 6BH. United Kingdom

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

Corresponding Author: Anna Hall (anna.hall@shu.ac.uk; 0114 2256279)

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/m^2) 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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Introduction

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

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

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

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

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

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

Methods

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

Study 1: Acceptability Study

As palatability can modulate food intake (Robinson *et al*., 2005; Yeomans *et al*., 2008), it is important to evaluate the sensory acceptability of test foods (Mattes *et al*., 2005). In this paper, the terms palatability and sensory acceptability have been used interchangeably similar to some previous studies (Archer *et al*., 2004; Killinger *et al*., 2004; Pelletier & Dhanaraj, 2006). Seventy nine untrained sensory panellists aged between 18 and 65 years (40 males, aged 18-65) were recruited to assess the sensory acceptability of 5 samples of wholemeal bread containing 0 g (control), 5 g, 10 g, 15 g and 20 g *Ascophyllum nodosum* (Seagreens® Ltd, West Sussex, UK) per 400 g loaf (Table 1).

Bread samples were toasted on each side for 1 minute, cut with a pastry cutter (7.5 cm diameter) to remove crusts and topped with scrambled eggs (prepared as described by McCance and Widdowson in The Composition of Food, Food Standards Agency and Institute of Food Research, 2002). Slice depth was kept constant using an industrial slicer. Samples were randomly coded using 3 digit blinding codes and were presented in a random order. In accordance with standard protocol (Mailgaard, Civille, & Carr, 2006), five sensory attributes (appearance, aroma, taste, texture, aftertaste), as well as overall acceptability were evaluated on touch screen operated visual analogue scales with extremes varying from extremely unacceptable (1) to extremely acceptable (9) using industry standard FIZZ software (Version 2.10c, Biosystemes, France). A score of 5 was used as a cut off for lower level acceptability (Mexis *et al*., 2010). A timed break of 1 minute was enforced between samples, during which panellists consumed water (≤200 mL, Brontë Natural Spring water LTD (UK)) and crackers (Carr's Water Biscuit, United Biscuits (UK) LTD) to cleanse their palates. Tests were conducted silently in temperature controlled (22-24°C) individual booths, with standardised 'natural' lighting, and positive-air flow. Results were analysed using one-way repeated measures ANOVA and Bonferroni *post-hoc* analyses on SPSS V17.0 (SPSS Inc. Chicago, USA).

Study 2: Satiety Study

12 males, aged between 18 and 65 years (Mean age 40.1 ± 12.5 years) self reported as overweight (BMI ≥25 kg/m^2) but otherwise healthy were recruited to take part in this study. Consistent with other research in the area of dietary fibre and appetite (Paxman *et al.*, 2008), the following exclusion criteria were applied to the study: individuals suffering from irritable bowel syndrome, inflammatory bowel disease, Cushing's syndrome, dumping syndrome, severe constipation, severe diarrhoea or coeliac disease, type 1 diabetes, food allergies or any serious medical condition. The study had a single blind, cross-over design. A wash out period of 1 week was considered appropriate in order to eliminate potential carry over effects.

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

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

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

At 8:30 on day 2, anthropometric measurements (as described previously) were taken and baseline capillary blood samples were collected from the finger tip using a single use Accu-chek® Softclix® Pro lancing device (Roche Diagnostics Ltd., West Sussex, UK). 30µL of blood was collected in Microsafe Collection and Dispensing Tubes (Inverness Medical, Cheshire, UK), applied immediately to the sample area of a Reflotron® Cholesterol Test Strip and inserted into the Reflotron® dry chemistry analyser. Total blood glucose was measured using a single droplet of capillary blood applied to a OneTouch® Ultra® Test Strip with FastDrawTM design which was inserted into a OneTouch® Ultra® Blood Glucose Monitoring System (reference range 1.1 to 33.3 mmol/L; Lifescan Inc., Bucks, UK). Participants were also asked to rate their baseline perceived hunger and fullness, along with 6 other 'distracter ratings' (“how friendly/ nauseous/ thirsty/ happy/ energetic/ relaxed do you feel?”) on 100 mm visual analogue scales (VAS) with left end points anchored at “not at all” and right end points anchored at “extremely”.

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

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

Over the subsequent 4 hours, an additional 10 capillary blood samples were taken (09.30, 09.45, 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 paper-based VAS questionnaires were completed following each blood collection. Blood samples were tested for glucose and cholesterol using procedures described earlier. Participants could drink ≤ 1 litre water *ad libitum* over the course of the morning. Water bottles were weighed prior to distribution and after 13:00 to quantify how much water had been consumed.

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

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

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

Results

Acceptability Results

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

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

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

Satiety Results

12 males were recruited for the satiety study (Age 40.1±12.5 years; BMI 30.8±4.4 kg/m^2). 1 participant was excluded from the dietary analysis due to incomplete diet diaries, and a different participant was excluded from cholesterol analyses as fasted blood results indicated hypercholesterolaemia (>6.5 mmol/L, Musial *et al*., 2001; Engbers, van Poppel, & van Mechelan, 2007).

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

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

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

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

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

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

Discussion

Ascophyllum nodosum enriched bread is acceptable

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

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

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

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

Ascophyllum nodosum enriched bread decreases energy intake at a test meal

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

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

Ascophyllum nodosum enriched bread has no effect on nutrient uptake

There were no significant differences in AUC glucose or cholesterol following the consumption of the *Ascophyllum nodosum enriched bread* compared to the control. Peak glucose values of 6.9mmol/l were reached at 75 minutes for both treatments and there were no significant differences in cholesterol levels at any time point throughout the intervention. In a small (n=14) yet well controlled pilot study, Paxman *et al*. (2008a) showed that compared to a control (containing no alginate), the consumption of a beverage containing 1.5 g sodium alginate significantly ameliorated the increased glucose and cholesterol uptake found in individuals with a higher body fat percentages compared to those with lower body fat percentages. Wolf *et al*. (2002) (n=30) also added alginate (3.6 g) to a beverage and while no difference was seen in peak glucose, a significant (p<0.01) decrease in AUC glucose was apparent when compared to the control. Torsdottir *et al*. (1991) saw a reduced rise in glucose (p<0.02) and a slower rate of gastric emptying (p<0.05) in 7 diabetic males following the consumption of 5 g sodium alginate compared to a control. Each of these studies used sodium alginate, a seaweed isolate, and suggested that the modulated glycaemic response was due to gelation of alginate causing a slower rate of gastric emptying and possible nutrient encapsulation. One study used whole red seaweed (Nori) in a capsule form (3 g) and measured the postprandial glucose response to white bread consumed 15 minutes later. The authors concluded that Nori seaweed significantly (p≤0.05) reduced AUC glucose, and again, postulate that delayed gastric emptying was the mechanism of action (Gõni *et al*., 2000). Previous studies have described how the inclusion of dietary fibre, may reduce blood cholesterol levels, and various mechanisms have been described (Braaten *et al*., 1994; Sola *et al*., 2010; Brown at al., 1999; Ripsin *et al*., 1992; Gunness, Flanagan & Gidley, 2008; Jeminez-Escrig & Sanchez-Muniz, 2000; van Horn *et al*., 1991; Behall *et al*., 2004). However it is evident that these benefits did not occur in the present study. Nutrient uptake in the current study was neither slowed nor reduced, suggesting neither gelation nor nutrient encapsulation occurred. A more likely mechanism here is that the seaweed acted as a bulking agent, increasing gastric stretch to a greater extent than standard wholemeal bread. It is also possible that an altered gut peptide response mediated enhanced satiety or brought about premature satiation at the subsequent test meal. The mechanism(s) of action for the observed effects warrant further investigation.

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

Ascophyllum nodosum enriched bread does not alter hunger and fullness ratings

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

Compliance

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

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

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

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Table 1: Ingredients in the control and enriched bread

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

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

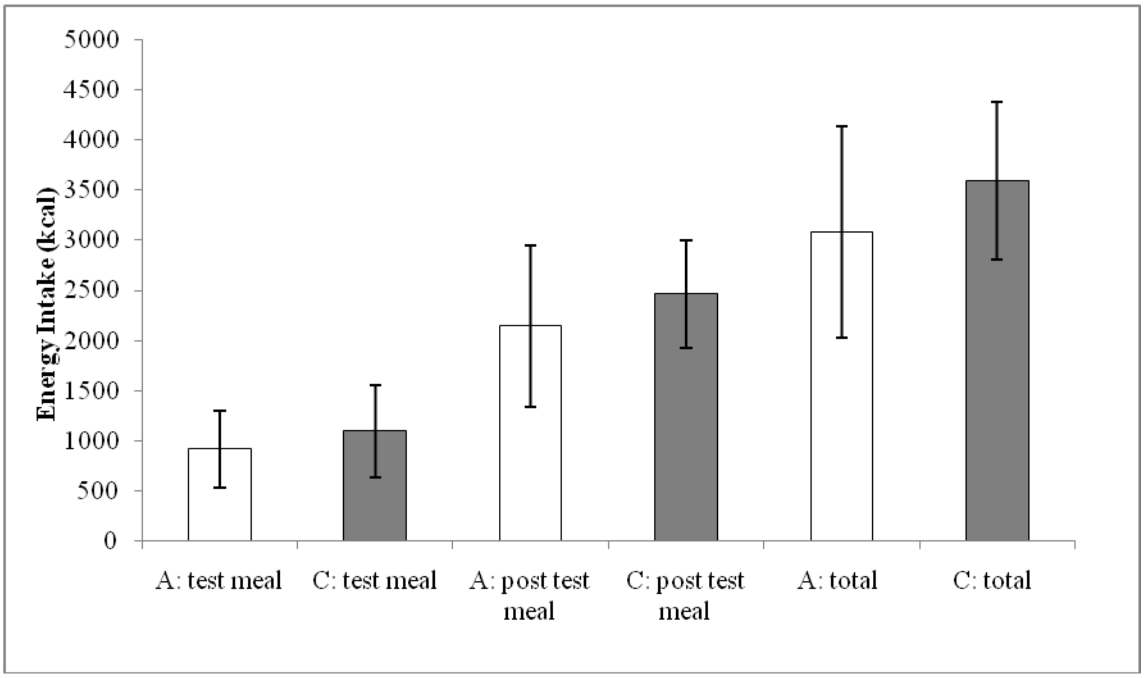
Amount of *Ascophyllum nodosum* per 400 g loaf (g)	**0**		**5**		**10**		**15**		**20**	
Estimated amount of alginate per 400 g loaf (g)	**0**		**1.15**		**2.3**		**3.45**		**4.6**	
	M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Appearance	6.42^{a}	1.80	6.46^{a}	1.58	6.41^{a}	1.38	6.58^{a}	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

Data are presented as means and standard deviations. Different letters in the same row denote means that are significantly different to one another (* p =.008, ¥ p=.003, §p=.002). Cut off for overall acceptability was 5 (Mexis et al., 2010).

Figure Legends

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

Figure 1



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

Figure(s)
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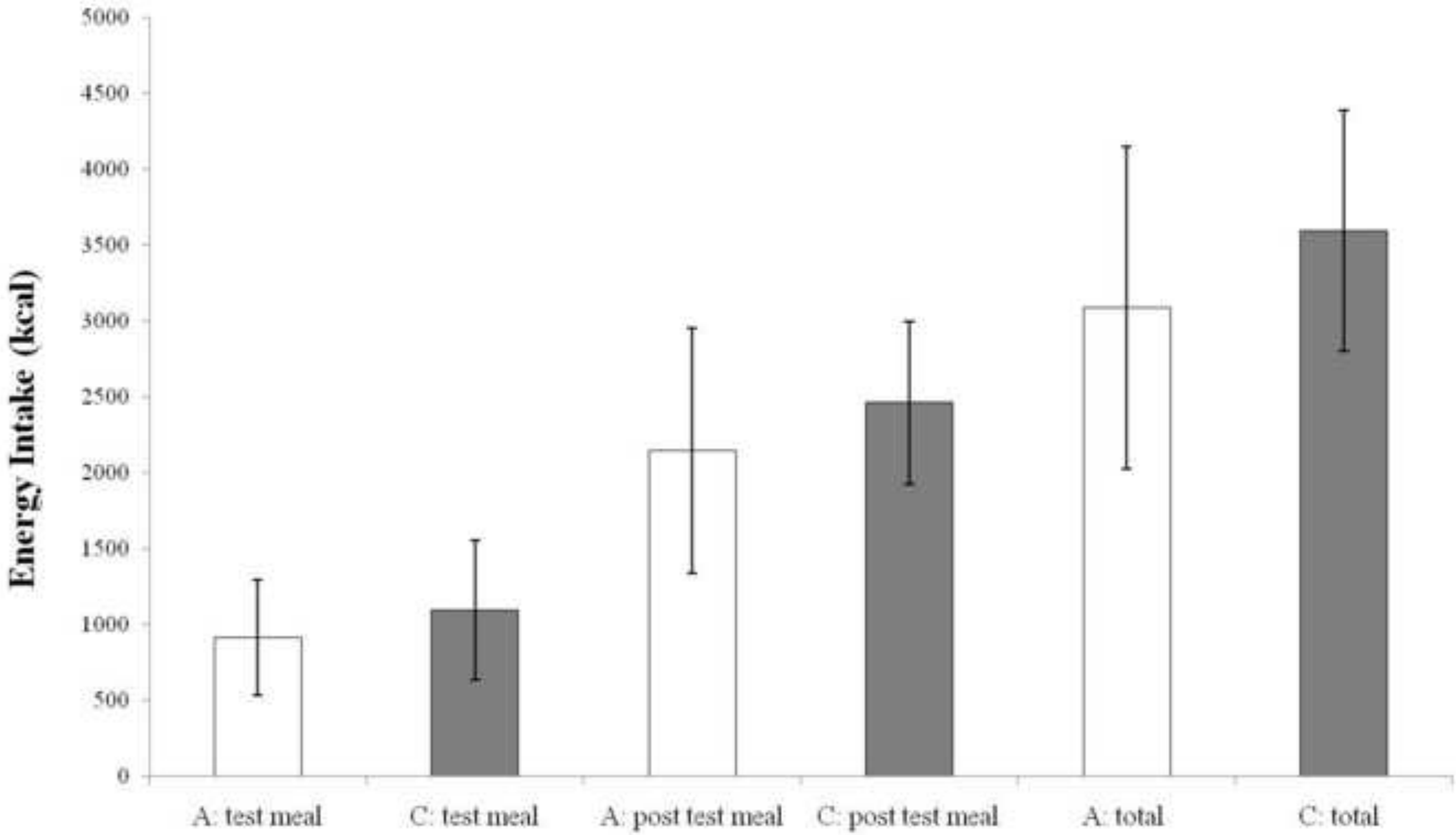

Figure 1