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Randomized Control Trials

Effects of brown seaweeds on postprandial glucose, insulin and appetite in humans – A randomized, 3-way, blinded, cross-over meal study

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SUMMARY

Background & aims: Seaweed including brown seaweeds with rich bioactive components may be efficacious for a glycaemic management strategy and appetite control. We investigated the effects of two brown edible seaweeds, *Laminaria digitata* (LD) and *Undaria pinnatifida* (UP), on postprandial glucose metabolism and appetite following a starch load in a human meal study.

Methods: Twenty healthy subjects were enrolled in a randomized, 3-way, blinded cross-over trial. The study was registered under ClinicalTrials.gov Identifier no. NCT00123456. At each test day, the subjects received one of three meals comprising 30 g of starch with 5 g of LD or UP or an energy-adjusted control meal containing pea protein. Fasting and postprandial blood glucose, insulin, C-peptide and glucagon-like peptide-1 (GLP-1) concentrations were measured. Subjective appetite sensations were scored using visual analogue scales (VAS).

Results: Linear mixed model (LMM) analysis showed a lower blood glucose, insulin and C-peptide response following the intake of LD and UP, after correction for body weight. Participants weighing $\leq$ 63 kg had a reduced glucose response compared to control meal between 40 and 90 min both following LD and UP meals. Furthermore, LMM analysis for C-peptide showed a significantly lower response after intake of LD. Compared to the control meal, GLP-1 response was higher after the LD meal, both before and after the body weight adjustment. The VAS scores showed a decreased appetite sensation after intake of the seaweeds. Ad-libitum food intake was not different three hours after the seaweed meals compared to control.

Conclusions: Concomitant ingestion of brown seaweeds may help improving postprandial glycaemic and appetite control in healthy and normal weight adults, depending on the dose per body weight.

Clinical trial registry number: Clinicaltrials.gov (ID# NCT02608372).

1. Introduction

Postprandial hyperglycaemia is characterised by a plasma glucose level >7.8 mmol/L (140 mg/dL) 2 h after ingestion of food. Normal fasting blood glucose levels are typically <6.1 mmol/L with 2-h postprandial plasma glucose <7.8 mmol/L (postprandial) [1]. Continued fasting and/or postprandial hyperglycaemia can lead to a progressive decline in hepatic and peripheral insulin sensitivity, deterioration of β-cells, and deficiencies in the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent gastric inhibitory peptide (GIP), secreted by the gut [2]. Restoring a normal

Abbreviations: C-peptide, Connecting peptide; CRM, Certified reference material; DPP-4, Dipeptidyl peptidase-4; GIP, Gastric inhibitory polypeptide; GLP-1, Glucagon-like peptide-1; iAUC, Incremental area under the curve; ICP-QQQ-MS, Inductively coupled plasma mass spectrometry; LD, *Laminaria digitata*; LMM, Linear mixed models; RSD, Relative standard deviation; UP, *Undaria pinnatifida*; VAS, Visual analogue scale.

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blood glucose level within a short interval after a meal is important for metabolic health, as hyperglycaemia may be a predecessor for type 2-diabetes (T2DM) development [3]. Furthermore, postprandial hyperglycemic events occur frequently in individuals with T2DM, varying between 61.9 and 88.4% of the patients experiencing hyperglycaemia once a week [4]. Therefore, minimising postprandial exposures to high blood glucose levels through dietary modification is one of the potential remedies for glycaemic management.

For centuries, seaweed has been consumed in Asian countries like China, Malaysia and Japan where it is a regular part of the daily diet. Elsewhere in the world, other seaboard countries such as Ireland, Scotland, Wales, Iceland, Norway, Canada and Spain have traditionally eaten seaweed but to a lesser extent [5,6]. Since ancient times, some seaweeds have been used in Asian countries as functional foods and medical herbs. Thus, the potential health benefits of seaweeds for human health are of interest [7].

Consumption of whole seaweed may provide a combination of bioactive components which may be more effective than the sum of the individual compounds in seaweed when tested individually [8]. Edible seaweeds are a particularly rich source of a variety of resistant dietary fibers, including xylans, carrageenan, fucoidan, laminaran and alginate. Some of these fibers are known to reduce glycaemia and insulin levels and some of them are known to improve satiety [9–11]. We previously showed that seaweed extracts from brown seaweeds efficiently inhibit starch-degrading enzymes *in vitro* and fibers as well as polyphenols and carotenoids were the responsible components [12,13]. Animal models demonstrate a positive effect on glucose metabolism after ingestion of some of the edible seaweed species [14,15]. However, there is a scarcity of human studies on the short- and long-term effects of seaweed on these endpoints. Therefore, the main aim of this study was to investigate whether the two brown seaweeds, *Laminaria digitata* (LD) and *Undaria pinnatifida* (UP), affect postprandial glucose, insulin, C-peptide, and GLP-1 concentrations in healthy adults. Moreover, we aim to evaluate the effect of these two seaweed species on subjective appetite sensation using visual analogue scales (VAS) and an *ad libitum* meal.

2. Materials and methods

2.1. Study design, ethics and protocol registration

The study had a randomized, 3-way, blinded cross-over design consisting of three test meals given in a random order to each participant on the different test days, which were separated by at least 7 days for a washout period. All of the participants were randomized by a balanced block design to one of the six possible sequences to receive the two test meals containing LD and UP or the energy-adjusted control meal containing pea protein at the three different test days, see Supplemental Fig. S1. The study was blinded to the investigator and to the statistician. The participants were not informed about the content of the test meal, but it was not possible to eliminate the seaweed taste; therefore they were semi-blinded. Participants were instructed to refrain from all kinds of seaweed and paracetamol 48 h prior to and throughout each test day, except for what was provided. In addition, the participants were instructed to refrain from caffeinated beverages including coffee, black, green, or white tea, cola, energy drinks and chocolate as well as alcohol during this same period. Furthermore, they were instructed to avoid intense physical activity 24 h proceeding each test day and until the following morning.

In the evening before each test day, the participants were fasting from 8 pm, but drinking 0.5 L of water was required between 6:00 pm and 08:00 am the next morming and again 0.5 L during the test day (08:00–12:00). For each test day, the participants had to meet, in a 12-h fasting state at 08:00. Upon arrival, participants' weight, height, and waist circumference were measured. They were instructed to lie down and rest for 10 min before the measurement of baseline blood pressure. A venflon catheter was afterward inserted into the antecubital vein, preferably of the right arm, allowing repeated blood sampling throughout the day.

The recruitment was carried out between May 2015–August 2015 at the Department of Nutrition, Exercise, and Sports, in the section for Preventive and Clinical Nutrition, University of Copenhagen, Denmark. The study protocol was approved by the municipal Ethical Committee of Copenhagen (journal no.: H 15004500) in accordance with the Helsinki-II declaration. All participants gave their written informed consent after having received written and oral information about the study. The study was registered on Clinicaltrials.gov (ID# NCT02608372). The primary outcome was the postprandial glucose response and the secondary outcomes were postprandial insulin response, C-peptide, GLP-1 and subjective appetite scores. The sample size was determined to obtain a statistical power of 80% at a significance level of $p < 0.05$ for $\geq$38 pmol/l change.

2.2. Participants

The study participants were recruited through posters at the University of Copenhagen and via website advertisement on http://www.forsogsperson.dk and www.sundhed.dk. Healthy, normal weight (BMI 20.5–25.0 kg/m^2) males and females aged between 20 and 50 years old were eligible for to the study. Exclusion criteria included, systemic infections, acute or chronic metabolic disorders, tobacco use, breastfeeding, pregnant or planning a pregnancy, were or had been drug addicts, iodine related intolerance or allergy, history of surgical intervention for treatment of obesity, had been enrolled in any human dietary or medical intervention study less than 4 weeks before the study, or habitual alcohol consumption above the maximal limit as recommended by the Danish Health Authorities (14 drinks per week for males or 7 drinks per week for females). All subjects were screened over the phone and invited to an information meeting, if they were qualified. Upon final enrollment, the subjects were randomized to one of the three test meals and the order in which they were to consume the test meal using the RAND function in in Microsoft Excel.

2.3. Test meals

The test meal was served in the morning at 08:45. A 150 mL starchy drink consisting of 30 g of corn starch in water with 22 g sugar free lemonade powder (Fun One, Stevia lemonade with guava/lime, Kavli A/S Hvidovre, Denmark) was served with either of three different meals. They consisted of 5.0 g of LA (obtained from AlgAran Teoranta, Kilcar Co. Donegal, Ireland) or 5 g of UP (obtained from JFC Deutschland, Dusseldorf, Germany) or 5 g of pea protein (Pea protein Mega 83%, Natur Drogeriet, Hørning, Denmark). We used whole seaweeds in the test meals as consumed by people in Asia, where seaweed salads with whole or chopped leafs are common. This provides minimal processing, thereby preserving the seaweed constituents. The dried seaweed were soaked in 200 mL of water for 10 min, then rinsed and drained to remove excess water. Finally, they were cut into pieces and added with 0.5 g iodine enriched salt (6.5 μg iodine). 0.2 g of black pepper and 4 g of fresh lemon juice were added to the test and control meals to improve the palatability. Together with the test meal, 500 mL of drinking water was additionally served. The minor differences in contents of carbohydrate, fat, and protein in the three meals were adjusted with corn starch, rapeseed and pea protein (83%). Table 1 shows an

overview of the nutrient composition and the energy content of the test meals.

Three hours after the test meal and immediately after the last VAS score participants were offered an *ad libitum* test meal to assess their hunger. The *ad libitum* meal consisted of 7987.6 kJ pasta with meat sauce, served with 250 mL water (energy: 554.5 kJ/100 g with macronutrient content (protein: 15.5 E %, carbohydrate: 54.5 E % and fat: 30.1 E %). The volunteers were given 30 min to complete this meal.

2.4. Biological sampling and analysis

The subjects had for each visit, seven separate blood draws by trained phlebotomists. Blood samples were collected at baseline −20 min and then at 20, 40, 60, 90, 120 and 180 min. Blood was collected for plasma glucose analysis in 3 mL FC-mixture tubes (VF−053SFC36, TERUMO Corporation, Tokyo, Japan) and for serum insulin, C-peptide and GLP-1 in 4 and 6 ml additive-free tubes (369032 and 366815 from Becton Dickinson, Plymouth, UK). Samples for plasma collection were centrifuged immediately after sampling while serum tubes were allowed to stand at room temperature for 20 min; serum and plasma were dispensed into cryotubes and subsequently frozen at −80 °C. Samples were thawed and assayed after they had all been collected. Plasma glucose was determined by a standard kit on an ABX Pentra 400 analyzer (Horiba ABX SAS, Montpellier, Cedex, France). Insulin and C-peptide was determined by solid-phase, two-site chemiluminescent immunometric assay, using Immulite 2000 XPi (Siemens Healthcare Diagnostic Ltd, Llaneris Gwynedd, United Kingdom). Serum concentrations of GLP-1 were determined using an ELISA based kit (Multi Species GLP-1 total ELISA, EZGLP1T-36K, EMD Millipore, Burlington, USA). The kit measures both the inactive and active form of GLP-1 (7-36- and 9-36amides) and was chosen based on the findings by Bak et al. [16].

2.5. Analyses of mineral elements

The concentration of selected mineral elements in the seaweed samples was determined following the principles in EN15763:2009 [17]. Briefly, subsamples of seaweed (approx. 0.3 g) were digested using 5 mL of concentrated nitric acid (SCP Science, Villebon-sur-Yvette, France) in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). Prior to analysis, the digests were diluted with milli-Q water and subsequently the total element concentration was determined using inductively coupled plasma mass spectrometry (ICP-QQQ-MS) (Agilent 8800, Agilent Technologies, Waldbronn, Germany). Quantification was done using external calibration with internal standardization. Analytical quality was assessed by running selected samples in duplicate (relative standard deviation (RSD) values in the range 1–20% for all elements) and by including the certified reference material (CRM) ERM-CD200 Bladderwrack [18] in the analytical run.

The content of iodine in the seaweed samples was determined following the principles in EN15111:2007 [19]. Briefly, subsamples of seaweed (approx. 0.3 g) were extracted using 4% tetramethylammonium hydroxide in an oven at 90 °C for 3 h. Prior to analysis the extracts were diluted with water and filtered and subsequently the iodine concentration determination using inductively coupled plasma mass spectrometry (Agilent 7500ce, Agilent Technologies, Waldbronn, Germany). Quantification was done at m/z 127 using external calibration with internal standardization with tellurium at m/z 125. Analytical quality was assessed by running selected samples (n = 3) in duplicate (RSD = 2.7%) and the use of the reference material CD200 Bladderwrack, where the obtained results was in good agreement with the target value for iodine established in a collaborative trial [20].

2.6. Measurements of subjective appetite sensations

Appetite registration was measured at all three test days by repeated visual analogue scales (VAS). VAS was used as a replacement for a categorical questionnaire to register scores for satiety, hunger, prospective food intake, fullness, comfort, and *ad libitum* energy intake as continuous variables. The first appetite assessment was carried out before consumption of the test meal (after 10–12 h fasting). Subjects were hereafter instructed to register VAS every approximately 20 min, following a guideline on a tablet screen, until the last registration at 180 min postprandially after the *ad libitum* meal. VAS was constructed as a digital horizontal line, equal to a 100 mm analogue line on a paper, with the question of interest set above the line. The extremes of the response options were indicated as vertical marks at each end of the line. The VAS equal to 0 and 100 mm is equivalent as follows: satiety ("I am completely empty" and "I cannot eat another bite"), hunger ("I am not hungry at all" and "I have never been more hungry"), prospective food intake ("How much do you think you can eat?" "Nothing at all" and "A lot"), fullness ("How full are you?" "I am totally full" and "Not full at all") and comfort ("How comfortable do you feel?" "Not comfortable at all" and "Very comfortable"). The participants were instructed to assess each question and mark with a vertical line presented on the tablet screen. The VAS registration was done using a digital tablet (Lenovo ThinkPad 10) running a VAS-assessment program, Acqui (Laugesen, J. L. at XYZT, Denmark, www.sensory.dk).

2.7. Statistical analyses

Formal power calculations were not possible since this study is the first to test the effect of seaweeds on postprandial glycaemia, however we have based the number of participants on previous comparable studies with coffees [21] or berries [22].

Statistical analysis was performed using RStudio software (version 1.0.153, ©2009–2016 Rstudio, Inc.) and R (version 3.4.1, R Core Team 2017). Figures were produced using the ggplot2 package

Table 1
Nutrient composition of test meals (g/serving).[a]

Nutrient composition	*Laminaria digitata*	*Undaria pinnatifida*	Pea protein, control
Energy (kJ)	502.7	536.6	518.9
Protein (g/serving)	0.8	1.1	1.0
Fat (g/serving)	0.3	0.2	0.3
Carbohydrate (g/serving)	27.3	29.2	28.8
Dietary fibre (g/serving)	1.8	1.7	0.2
Water (g)	206.7	206.7	206.7

[a] Proximates of the test meals foods where obtained from the Dankost PRO software [43] and from analysis performed by a ISO 17025 accredited laboratory (Eurofins, Vejen, Denmark).

[23]. The descriptive data are presented as mean ± SD, ±SEM. All variables were checked for outliers and missing data. Dependent variables were inspected for homogeneity of variance and normal distribution using plots of residuals and normal probability. Non-normally distributed data were logarithmically transformed and reassessed for normal distribution before further analysis. Glucose, insulin, C-peptide, GLP-1 responses, and VAS scores were calculated as the incremental area under the curve (iAUC) from baseline values. Blood concentrations of glucose, insulin, C-peptide and GLP-1 are presented as mmol/L, pmol/L, pmol/L and pmol/L respectively. Data for VAS questions are shown as mm within the range of 0–100 mm. R lme4 package [24] was used to perform a linear mixed model (LMM) analysis using ANOVA for repeated measures on all outcomes, with time, treatment, and body weight as fixed effects and subject, sex, and visit (randomization order) added as random effects. Tukey's post hoc test for pairwise comparisons were performed using R multcomp [25], at each time point with time 0 as a co-variate if the model showed statistical significance. The same procedure was applied to all VAS questions. All 2-sided *p*-values < 0.05 were considered statistically significant.

3. Results

3.1. Participant characteristics

Forty subjects were screened and 20 healthy subjects (9 males and 11 females) were recruited. All volunteers completed the study, which gave a dropout rate of 0% (Fig. 1). The baseline characteristics of the subjects are shown in Table 2.

3.2. Nutrient content and mineral elements

The nutrient and mineral contents varied between the two species, LD and UP. In general, both of them had approximately 1.5 g higher dietary fibre content (Table 1) compared to the control meal. Table 3 shows an overview of the selected mineral contents of the tested seaweeds. Furthermore LD also has 5 times higher iodine content and almost 20 times higher K/Na ratio than UP (Table 3).

3.3. Blood glucose, insulin, C-peptide and GLP-1 response

There were no differences from control in iAUC for glucose, log-transformed insulin or C-peptide after intake of any of the two seaweed meals (Table 4). However, glucose, insulin and C-peptide response was changed when including body weight in statistics by using a 3-factor interaction model meal * time * body weight (Table 4). In order to illustrate this effect of body weight graphically we performed a sub-analysis by weight using the participants' median weight, 63 kg as a cutoff. The two weight groups; $\leq$ 63 kg (n = males:0, females:10) and >63 kg (n = males:9, females:1) did not differ by BMI (median BMI was 21.15 kg/m^2 for $\leq$ 63 kg group and 21.39 for > 63 kg group) and consequently differed by height, but showed no difference in fasting glucose or insulin at baseline. Participants weighing $\leq$ 63 kg had a reduced glucose response compared to control meals at 40, 60 and 90 min both after LD ($P = 0.02$, $P = 0.001$, and $P = 0.004$, respectively) and UP ($P = 0.04$, $P = 0.02$, $P = 0.01$, respectively) meals. Participants weighing $\geq$63 kg had a reduced glucose response after meals with LD at 120 min ($P = 0.04$) compared to control (Fig. 2 and Supplemental Table S1). The effect of weight was independent of sex (Supplemental Table S2).

The insulin data was log transformed before the analysis. After adjustment for body weight, seaweed ingestion resulted in a lower postprandial insulin response, particularly between 20 and 60 min (Table 4 and Supplemental Fig. S2). Females had a lower insulin response at 20 min ($P = 0.004$) after UP treatment. After LD ingestion, males had a lower insulin response at 20, 40 and 60 min ($P = 0.03$, $P = 0.003$, $P = 0.05$ respectively), compared to control (Supplemental Table S3). iAUC for C-peptide was significantly higher in control, only after the correction for body weight (Table 4). C-peptide secretion was overall lower after the LD meals ($P = 0.02$) compared to control (Supplemental Table S4 and Supplemental Fig. S3). The iAUC for GLP-1 was higher after intake of LD when compared to control ($P = 0.017$). The LMM analysis, revealed an overall increase of GLP-1 for LD compared with control ($P = 0.05$). Thus, the GLP-1 secretion was increased at 120 min ($P = 2.2e\text{-}05$) after intake of the LD meal compared to the control meal (Fig. 3 and Supplemental Table S5).

3.4. Appetite and comfort scores

The postprandial changes in satiety, hunger, fullness, anticipated prospective food consumption, comfort and *ad libitum* energy are shown in Table 4. Satiety and fullness iAUC were higher after intake of UP ($P = 0.0002$ and $P = 0.008$, respectively) and LD ($P = 0.032$ and $P = 0.001$, respectively) compared to control. More precisely the subjects felt more satiated after ingesting UP, at 20 min ($P = 0{,}0030$) and percived an increased sensation of fullness after intake of the UP meal at 20 min ($P = 0.0002$) and at 50 min ($P = 0.01$) and after the LD meal at 20 min ($P = 0.041$ in comparison to the control meal (Fig. 4). There were no differences in iAUC between the three test meals for scores of hunger and anticipated prospective food consumption. However, hunger was reduced after the UP meal at 20 min ($P = 0.0005$) and subjects had a reduced desire to eat after the UP test-meal at 20 min ($P = 0.001$), 40 min ($P = 0.02$), 50 min ($P = 0.01$), 70 min ($P = 0.04$) and at 100 min ($P = 0.05$), compared to control (Fig. 5). The effects on the appetite scores did not remain after 200 min and comfort scores did not change at any time. The *ad libitum* energy intake at 200 min was not different between the test meals.

4. Discussion

Our findings suggested that the brown seaweeds, LD and UP, may reduce postprandial plasma glucose in healthy adults after a starchy meal. However, the effect is only seen when body weight is included in the statistical model, indicating that the effect of a fixed dose of seaweed decreases with increased body weight. The postprandial serum insulin response was lower after the consumption of seaweeds. LD resulted in a lower iAUC for C-peptide and a higher iAUC for GLP-1.

Brown seaweeds contain potentially bioactive compounds that inhibited α-amylase and α-glucosidase enzymes *in vitro* and reduced blood glucose and plasma insulin concentrations in mice [26,27]. Polyphenols, fucoxanthin and fatty acids found in LD and UP inhibit α-glucosidase activities resulting in a reduced rate of glucose liberation thereby reducing the postprandial rise in blood glucose. Other nonpolar components including oleic acid and linoleic acid found in LD also seem to inhibit α-glucosidase *in vitro* [28]. Crude water extracts from different brown seaweeds strongly inhibit the enzyme Dipeptidyl peptidase-4 (DPP-4) and stimulate GLP-1 and GIP secretion *in vitro* [29]. Furthermore, various organic extracts from other Sargassum species (S. polycystum and *S. wightii*) exhibit similar properties *in vitro* [30,31]. The results from our study provide additional evidence that relatively small dietary intakes of whole seaweed may affect postprandial plasma glucose, serum insulin and GLP-1 concentrations in healthy human adults.

High contents of dietary fiber in LD and UP may lead to reductions in blood glucose and insulin concentrations [10]. However

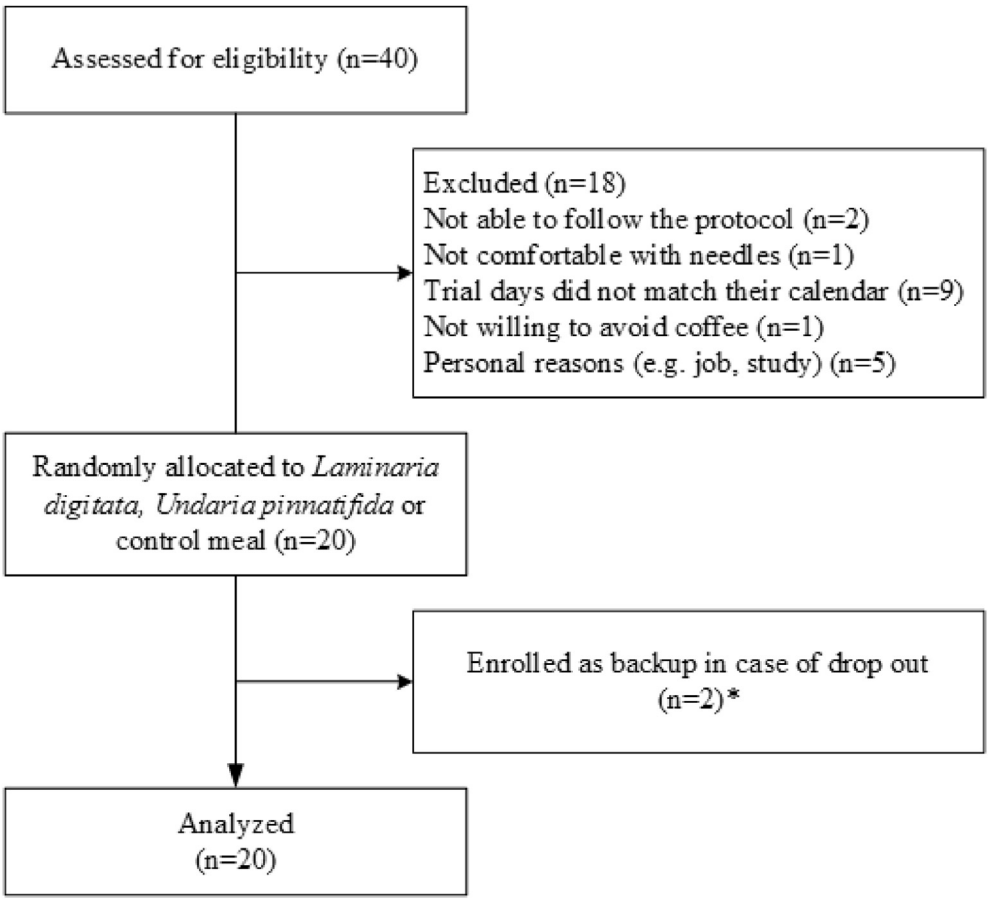

Fig. 1. Participant flow chart. * Not included in any analysis.

Table 2
Subjects characteristics at baseline.[a]

Subject characteristics	All ($n = 20$)	Males ($n = 9$)	Females ($n = 11$)
Age (y)	28.8 ± 5.4	30.3 ± 7.1	27.5 ± 3.4
Height (cm)	171.3 ± 14.5	183.6 ± 10.0	160.7 ± 7.7
Weight (kg)	63.6 ± 11.5	73.2 ± 9.7	55.9 ± 5.4
BMI (kg/m^2)	21.4 ± 2.2	21.6 ± 1.2	21.7 ± 1.9
Waist circumference (cm)	76.4 ± 8.0	81.0 ± 6.7	71.8 ± 6.9
Blood pressure, systolic (mm Hg)	110.3 ± 0.9	112.5 ± 1.5	108.3 ± 0.4
Blood pressure, diastolic (mm Hg)	67.8 ± 0.2	63.6 ± 0.6	70.6 ± 0.6
Fasting blood glucose mmol/L	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.5
Fasting blood insulin pmol/L	45.5 ± 26.1	39.3 ± 17.3	50.6 ± 30.9
Fasting blood GLP-1 pmol/L	17.9 ± 7.0	17.5 ± 4.9	18.3 ± 8.3
HOMA-IR	1.78 ± 1.0	1.56 ± 0.7	1.97 ± 1.1

[a] Numbers represent mean ± SD.

Table 3
Content of selected minerals in soaked, blotted *Laminaria digitata* and *Undaria pinnatifida*.

Mineral composition	*Laminaria digitata*	*Undaria pinnatifida*
Arsenic (As) (mg/100g)	4.00	5.81
Cadmium (Cd) (mg/100g)	0.018	0.137
Chromium (Cr) (mg/100g)	0.048	0.096
Iodine (I) (mg/100g)	164.6	32.0
Zinc (Zn) (mg/100g)	5.53	4.00
Calcium (Ca) (g/100g)	2.01	1.27
Magnesium (Mg) (g/100g)	0.683	0.406
Potassium (K) (g/100g)	1.46	0.275
Sodium (Na) (g/100g)	2.77	5.46

such effects are usually seen only at higher fiber intake levels than the 1.8 g provided in our study [32]. Brown seaweeds contain between 19.6 and 64.9% of soluble fiber depending on the species [33] and the fiber content of around 40% observed for our batches is therefore not extraordinary. The soluble fiber from seaweed dissolves in water to form a viscous gel [33], which might lead to a reduced rate of gastric emptying [33] or simply a reduced substrate diffusion resulting in a lower intestinal glucose absorption rate. Alternatively, glucose liberation from degradable polysaccharides such as starch may be delayed after entering into the duodenum thereby retarding the rate of glucose reaching the intestinal contents when seaweeds are consumed together with a meal rich in starch.

Table 4
Result for all measured endpoints after ingestion of the three test meals.

Outcome	Control *Total iAUC (n = 20)*	*Laminaria digitata* *Total iAUC (n = 20)*	*Undaria pinnatifida* *Total iAUC (n = 20)*	*P* - value *iAUC*	*P* - value LMM_{rm}
Biochemistry					
Glucose (mmol/L)	135.9 ± 76.8	105.4 ± 45.4	104.7 ± 80.1	0.24	0.003**
Insulin (pmol/L)	11606 ± 8989	9552 ± 6954	10095 ± 7377	0.28	0.04*
C-peptide (pmol/L)	77579 ± 48812	65921 ± 37820	67430 ± 41563	0.08	0.04*
GLPx1-1 (pmol/L)	196.2 ± 239.9	370.2 ± 261.9	250.6 ± 243.8	0.02*	0.05*
Appetite scores					
Satiety (mm)	642 ± 886	1315 ± 1399	1919 ± 1810	0.004 **	0.009**
Hunger (mm)	2138 ± 1837	1098 ± 1192	1509 ± 2351	0.18	0.02*
Fullness (mm)	806 ± 705	1405 ± 1323	2074 ± 1746	0.008 **	0.02*
Prospective food-consumption (mm)	1675 ± 1236	1204 ± 1631	1161 ± 1488	0.32	0.03*
Comfort (mm)	1191 ± 1613	916 ± 1155	1179 ± 1586	0.55	0.81
Ad libitum[a] (g)	629 ± 312	545 ± 251	627 ± 277	NA	0.13[a]

Comparison of iAUC, incremental AUC for all outcomes and LMM_{RM} linear mixed model repeated measures with outcome ~ meal *time*weight + visit, as the dependent variable. The fixed effect "meal" is presented as mean ± SD. *P* - values are obtained by gradually reducing the full model to a model showing a difference relevant to the outcome. In case of no difference, the p-value for the full model is displayed. The significant codes are: *** for 0.001; ** for 0.01 and * for 0.05. NA; not applicable; the *ad libitum* food intakes could be compared using a linear mixed-effect model only.

[a] Presented as mean gram of the eaten meal.

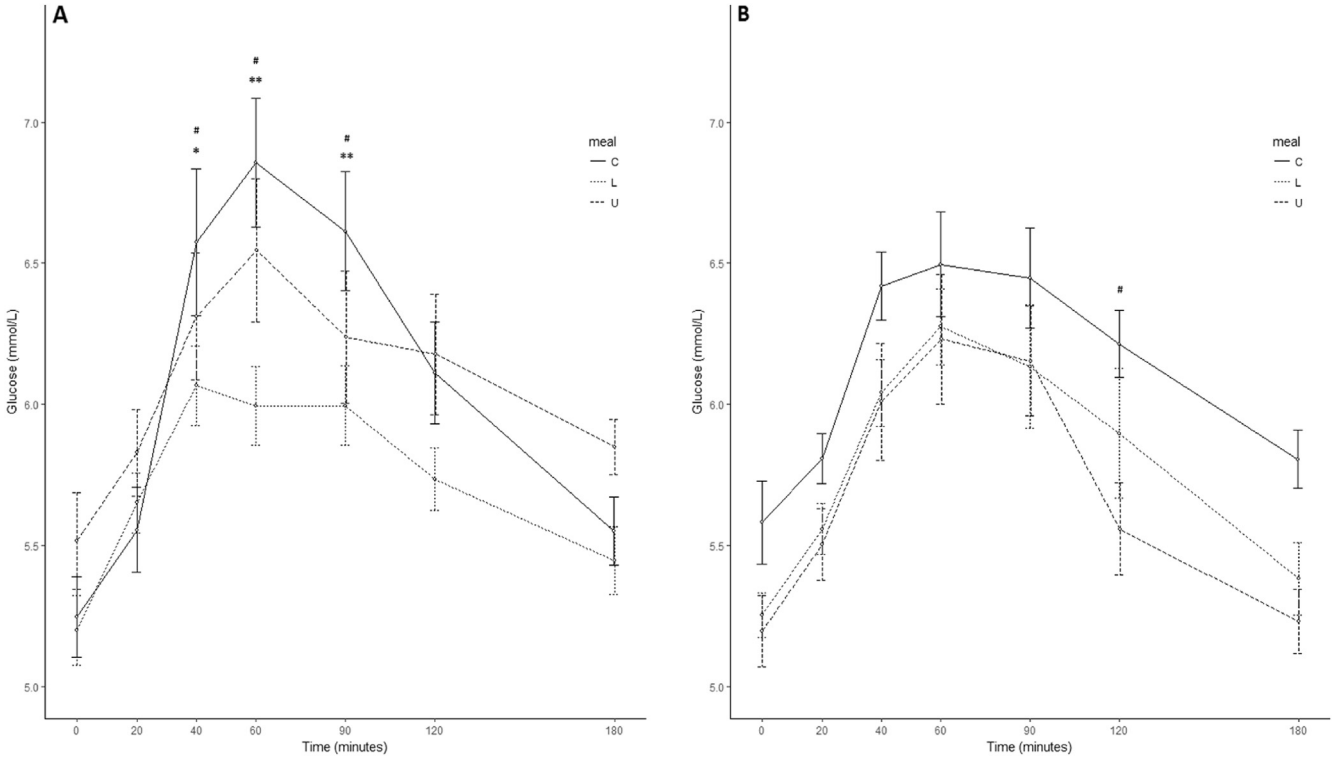

Fig. 2. Effects of Laminaria digitata and Undaria pinnatifida on postprandial plasma glucose concentrations (mmol/L) in subjects weighing < 63 kg (A) and subjects weighing ≥ 63 kg (B) at 0, 20, 40, 60, 90, 120 and 180 min after the intake of control and seaweed meals. C = control meal, L = *Laminaria digitata* and U = *Undaria pinnatifida*. Values are represented as means ± SEM, (n = 10). Significant symbols: '*' = *Laminaria digitata*, '#' = *Undaria pinnatifida*.

The lower postprandial blood glucose concentrations after the intake of LD and UP observed in our study is probably a dose dependent effect. By adding body weight as a fixed effect in the LMM model, it appears that weight had an influence on the postprandial glucose response after the test meals. The subjects weighing <63 kg had a significantly lower glucose response after both LD and UP especially pronounced between 40 - 90 min (Supplemental Table 1). This could be explained to some extent by the weight span between the enrolled subjects (48.5–93 kg), suggesting that there may be a dose-dependent effect of LD and UP.

The average fasting concentration of GLP-1 were lower than the postprandial GLP-1 concentrations at time 20 min after all test meals with LD, UP, or control. The increment correlated with commonly observed peak-response approximately 30 min postprandially [34,35]. However, as no data is available from baseline to 20 min and at 60 min, it cannot be ruled out that the C_{max} of GLP-1 was higher or occurred before or after time 20 min. GLP-1 concentrations were significantly different at 120 min after intake of LD compared to control. We speculate that the time course for laminarin to inhibit DPP-4 is delayed because of the viscous dietary fibre load from LD causing a delayed absorption of the meal and as a result, an increase in GLP-1 at time 120 min.

LD and UP also increased the sensation of satiety and reduced any feelings of hunger. As opposed to the lack of effects of pea

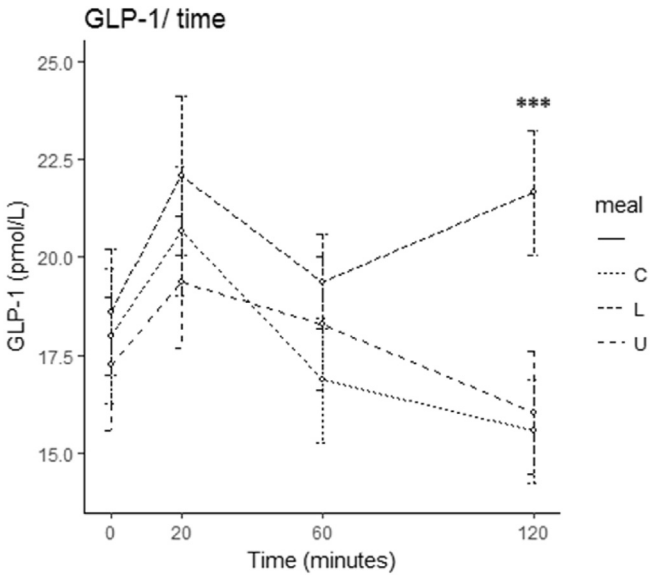

Fig. 3. Effects of *Laminaria digitata* and *Undaria pinnatifida* on postprandial plasma GLP-1 concentrations picomoles per litre (pmol/L) at 0, 20, 60 and 120 min after the intake of control and seaweed meals. C = control meal, L = *Laminaria digitata* and U = *Undaria pinnatifida*. Values are represented as means ± SEM (n = 18). Significant symbols: * *Laminaria digitate*.

seaweed is linked to increased satiety by delaying gastric clearance, stimulating gastric stretch receptors, and attenuating nutrient absorption [11].

The analysis of nutrient and mineral composition show that LD and UP contained some protein but only small amounts of fat. Both brown seaweeds, and especially LD, were rich in minerals such as potassium (K), calcium (Ca) and magnesium (Mg), which are proposed to improve glycaemic control [38]. Both LD and UP also contained zinc (Zn) and chromium (Cr) in relatively high amounts, which are associated with improved circulating glucose levels [39,40]. LD and UP contained high amounts of iodine so caution may be needed in case of frequent consumption as 0.36 g LD and 1.9 g UP would exceed the recommended upper tolerable level of daily intake at 600 μg/day for adults [41]. Despite the high iodine content in the test meals, the acute exposure are considered safe for subjects who are not hypersensitive to iodine since single acute doses from foods are not known to give adverse effects [42].

The study has several strengths. The food intake is fully controlled and the cross-over design assures individual control of effects. Also, the follow-up periods are fully monitored and supervised providing good assurance for validity of the measurements. Our study is novel, being the first to study the effects of common edible brown seaweeds in humans. The species were selected based on their ability to inhibit the starch degrading enzymes, alpha-amylase and alpha-glucosidase *in vitro* [12,13]. This provides a

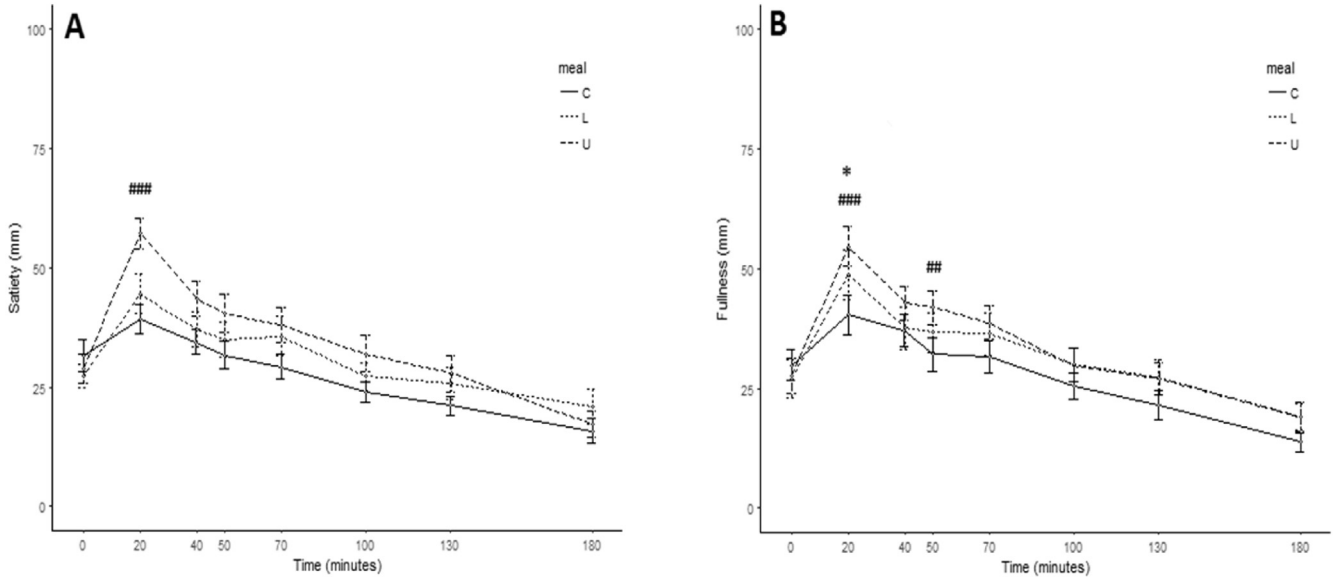

Fig. 4. Effects of *Laminaria digitata* and *Undaria pinnatifida* on postprandial perception of satiety (A) and hunger (B) at 0, 20, 40, 60, 70, 100, 130 and 180 min after the intake of control and seaweed meals. C = control meal, L = *Laminaria digitata* and U = *Undaria pinnatifida*. Values are represented as means ± SEM (n = 20). Significance symbols: * *Laminaria digitata*, #*Undaria pinnatifida*.

protein on postprandial measures of satiety [36] our findings show that consumption of brown seaweed affected subjective satiety and hunger. From the corresponding VAS questions, it appears that intake of just 5 g of whole, dried LD and UP affect several appetite related feelings for more than 1 h postprandially and satiety for more than two hours. This implies that these seaweed species are potential candidates to reduce energy intake for several hours after intake of dried LD and UP. The effect is possibly due to their content of the polysaccharide, alginate, and perhaps other soluble dietary fibers having a satiating effect that may be caused by bulking or to reduced gastric emptying rate [37]. Previously, alginate from brown

rationale for their effects on blood glucose as well as identification of the components potentially providing the bioactivity.

The study also has some weaknesses. The control meal contained only a limited amount of pea fiber. Pea protein (10 g) combined with pea hull fiber (7 g) is well known to have effects on postprandial blood glucose and this effect was not reduced by adding insoluble hull fiber [36]. Therefore, our use of 5 g pea protein as control represents a low dose of another bioactive anti-glycaemic meal component and may therefore have partially masked an effect of the seaweeds in this study. We attempted to blind the subjects, yet it was not possible to eliminate the taste

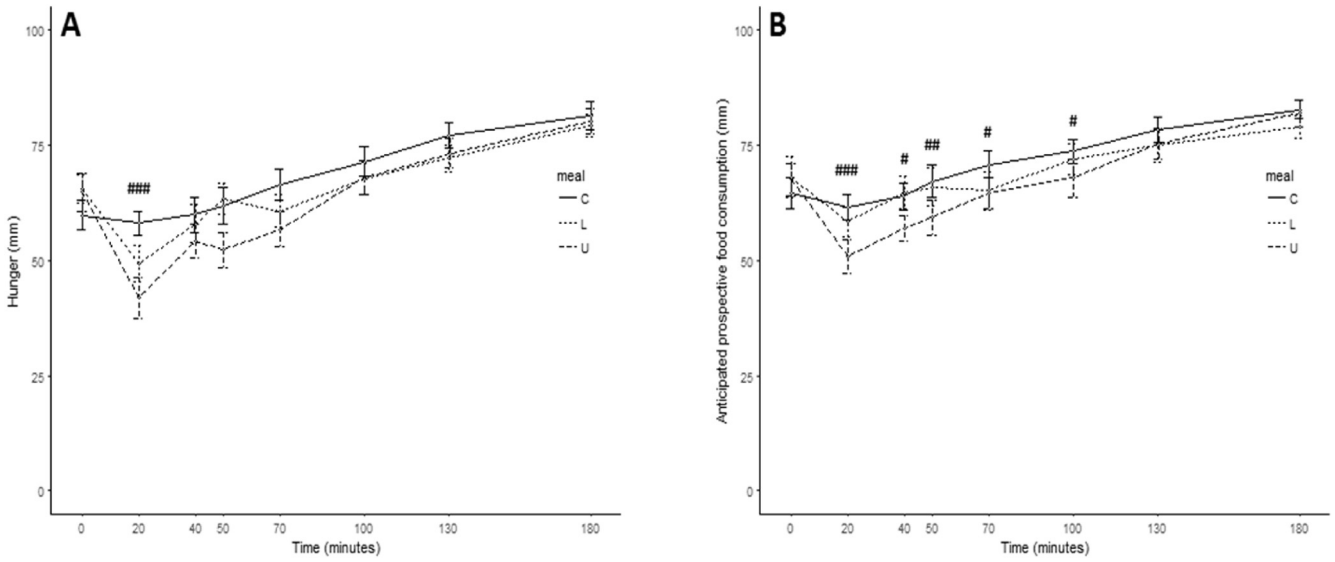

Fig. 5. Effects of *Laminaria digitata* and *Undaria pinnatifida* on postprandial feellings of hunger (A) and anticipated prospective food consumption (B) at 0, 20, 40, 60, 70, 100, 130 and 180 min after the intake of control and seaweed meals. C = control meal, L = *Laminaria digitata* and U = *Undaria pinnatifida*. Values are represented as means ± SEM (n = 20). Significance symbols: * *Laminaria digitata*, #*Undaria pinnatifida*.

differences of the control and seaweed meals. Thereby, VAS scores might be affected from the participants' awareness of the tested meal. Since this is only a meal study it provides no information on longer-term effects of seaweed. Finally, clinical significance of a single dietary exposure is limited for achieving the glycaemic control.

Further studies on isolated bioactive compounds from seaweed and a longer-term study with different groups of volunteers such as healthy -or hyperinsulinemic subjects are needed in order to see the individual contributions of seaweed components to the satiating effects and to glucose/insulin maintenance over time. In addition, further work is needed to identify the bioactive compounds, longer-term effects, mixed-meal effects, and mineral bioavailability in humans after LD and UP intake.

5. Conclusions

This study provides human trial evidence for an effect of brown seaweeds on glycemic and insulinemic responses, GLP-1 secretion, and appetite. Brown seaweed lowers the postprandial glucose and insulin response as well as hunger in humans exposed to a highly degradable linear starch and increased the postprandial feeling of satiety and fullness in healthy subjects of both sexes. Consumption of brown seaweed may be recommended as part of the diet for people with hyperglycaemic disorders, provided issues with potential excessive iodine intake can be avoided.

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Data sharing

Data described in the manuscript, code book, and analytic code will be made available upon request. Data sharing will take place through the new DASH-IN system which is currently under development at NEXS, University of Copenhagen.

Authors' contributions

NZ, MT, and LOD designed and conducted the research. MT performed the statistical analyses. JJS and RRR conducted mineral analyses. MT and CTP drafted the manuscript. All authors read and reviewed the manuscript and approved the final version.

Conflict of interest

NZ, MT, CTP, JJS, RRR, LOD declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.08.027.

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